

Toxicology and Carcinogenesis Studies in Haploinsufficient p16^{lnk4a} /p19^{Arf} N2 Mice (B6.129-Cdkn2a^{tm1Rdp})

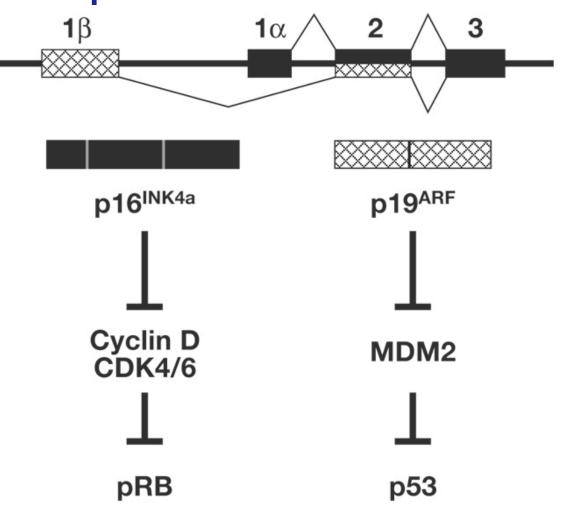
Benzene - GMM 8



Study Rationale Evaluation of Mouse Models

- NTP Evaluation of Genetically Altered Mice
 - Chemical hazard identification
 - Shorter study time (<2 yrs)
 - Fewer animals per group
- Multisite carcinogens selected for evaluation
 - Benzene
 - Glycidol
 - Phenolphthalein

INK4a/ARF Locus (CdKn2a gene): codes for two proteins

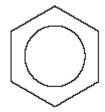


Haploinsufficient p16^{INK4a}/p19^{Arf} Studies

Haploinsufficient p16lnk4a /p19Arf Mice

- Mice placed on study at 5-6 weeks of age
- 80% C57BL/6, 19% 129/Sv, 1% SJL
- Mouse derived from Serrano and DePinho mouse
 - Haploinsufficient p16^{+/-} mouse (Serrano et al, Cell, 85:27-37,1996)

Haploinsufficient p16^{lnk4a} /p19^{Arf} Mice Benzene Studies



Benzene CAS No. 71-43-2

- Used as a solvent in chemical and pharmaceutical industries
- Starting material in synthesis of other chemicals
- Found in petroleum products
- 7.2 million metric tons of benzene produced in the U. S. in 2002
- IARC Group I chemical carcinogenic to humans
- NTP Report on Carcinogens known human carcinogen



NTP Studies: Benzene Multisite Carcinogen

Benzene TR 289 (oral gavage study) - F344 rat and B6C3F1Mouse

Male Rat Zymbal gland Oral cavity Skin

Female Rat Zymbal gland **Oral Cavity**

Male Mouse Zymbal gland Lymphoma Lung Harderian gland Preputial gland

Lymphoma Lung Harderian gland Mammary gland Forestomach Forestomach

Female Mouse

Zymbal gland

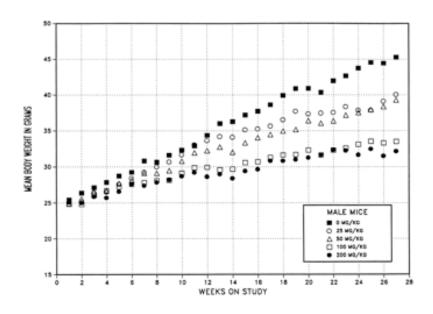
Ovary Liver

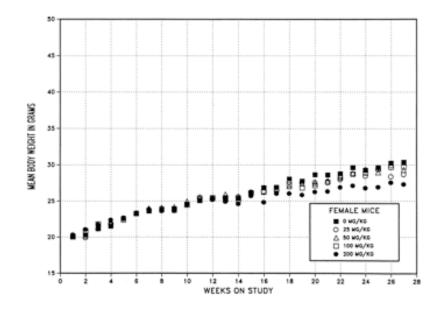
Positive in male p53(+/-) mice - lymphoma and histiocytic sarcoma

Dose Selection Benzene Haploinsufficient p16^{lnk4a} /p19^{Arf} Mouse Study

- Doses selected to overlap NIEHS/NTP benzene study doses
 - NTP 2-year B6C3F1 mouse study
 - 0, 25, 50, 100 mg/kg
 - NIEHS 26-week p53(+/-) mouse study
 - 0, 100, 200 mg/kg
- NTP 27-week haploinsufficient p16^{INK4a}/p19^{Arf} mouse study
 - 0, 25, 50, 100, 200 mg/kg
 - 15 animals/dose/sex
 - Oral gavage in corn oil

Body Weight - Male and Female Mice





Benzene: Males - Selected Non-neoplastic Lesions

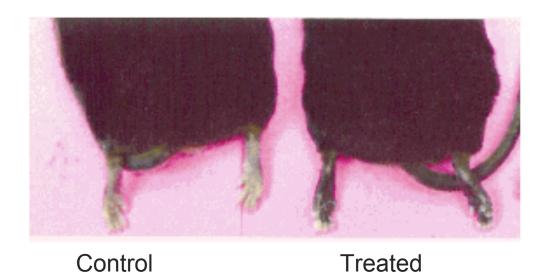
Dose (mg/kg)	0	25	50	100	200
Bone Marrow: Atrophy	0/15	0/15	0/15	10/15**	12/15**
Thymus: Atrophy	0/15	0/15	0/15	7/15**	13/15**
Lymph Node: Atrophy mesenteric	1/15	2/15	2/14	13/15**	13/15**
Spleen: Hematopoietic cell proliferation	0/15	1/15	0/15	2/15	8/15**
Skin: pigmentation	0/15	15/15**	15/15**	15/15**	14/15**

^{**}p ≤0.01

Benzene: Females - Selected Non-neoplastic Lesions

Dose (mg/kg)	0	25	50	100	200
Lymph Node: Atrophy mesenteric	0/15	2/15	3/15	8/15**	6/15**
Skin: pigmentation	0/15	1/15	8/15**	15/15**	15/15**

Benzene-induced Skin Pigmentation



Benzene: Males - Selected Hematology Results

Dose (mg/kg)	0	25	50	100	200
Hematocrit (%)	51.5	50.2	47.3	44.3**	40.9**
Hemoglobin (g/dL)	17.1	16.4**	15.4**	14.6**	13.4**
Erythrocytes (10 ⁶ /μL)	11.5	10.7**	10.0**	9.1**	8.3**
Leukocytes (10 ³ /μL)	4.2	2.8*	2.4**	1.4**	0.9**
Lymphocytes (10 ³ /μL)	3.7	2.3*	1.9**	1.0**	0.6**

^{*}p ≤0.05 **p ≤0.01 hematology measurements at 13 weeks

Benzene: Females - Selected Hematology Results

Dose (mg/kg)	0	25	50	100	200
Hematocrit (%)	50.1	50.2	48.8	47.4*	46.7**
Hemoglobin (g/dL)	17.0	16.7	16.4	15.8**	15.7**
Erythrocytes (10 ⁶ /μL)	10.9	10.8	10.5*	9.9**	9.8**
Leukocytes (10 ³ /μL)	5.1	4.8	3.6*	3.3**	3.8*
Lymphocytes (10 ³ /μL)	4.5	4.4	3.1*	2.8**	3.0**

^{*}p ≤0.05 **p ≤0.01 hematology measurements at 13 weeks

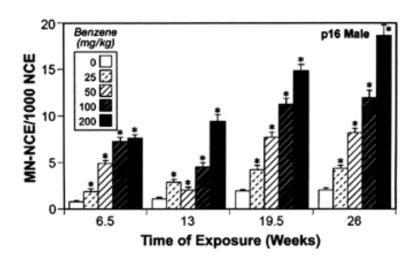
Benzene: Males - Neoplastic Lesions

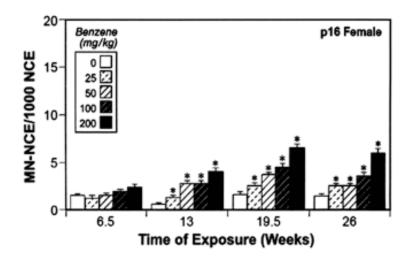
Dose (mg/kg)	0	25	50	100	200
Malignant Lymphoma	0/15**a	0/15	0/15	0/15	5/15 (33%)*

*p = 0.021 ** $p \le 0.001$

^aTrend Statistic:

Benzene - Micronucleus Findings





Benzene Conclusions

- Clear evidence of carcinogenic activity in male haploinsufficient p16^{lnk4a} /p19^{Arf} mice
 - Malignant lymphomas
- No evidence of carcinogenic activity in female haploinsufficient p16^{lnk4a} /p19^{Arf} mice
- Treatment related toxicity to hematopoietic system, lymphoid atrophy, and pigment in extremities